Amendments to the Specification

Please amend the Specification as follows.

At pages 4-5 of the filed Specification, replace paragraph [0014] (corresponding to paragraph [0019] of the published application) with the following new paragraph.

Disintegration of the cells can be achieved by physical, chemical or enzymatic methods. Most of currently available procedures were developed for the release of proteins from the cells and can not be used for polynucleotides without certain adaptations. Limitations of the established techniques are due to the differences of the physico-chemical properties between proteins and polynucleotides. High-pressure homogenization, the most common technology for the recovery of proteins, cannot be used for polynucleotides due to their size-depending shear force sensitivity and possible destruction of gDNA. (Carlson A, Signs M, Liermann L, et al. (1995) Biotechnol Bioeng 48:303). Chemical (Foster D (1992) Biotechn 10, (12):1539) and enzymatic (Asenjo JA, Andrews BA (1990) Bioprocess Technol 9: 143) methods cause minimal mechanical stress and minimal irreversible deterioration of the plasmid. Since it is the gentlest method, enzymatic disintegration utilizing lysozyme is the method of choice on laboratory scale. Typically, lysozyme is animal-derived (most commonly from chicken egg white) and therefore its use is a potential health risk (prions) and is considered as problematic by regulatory authorities like FDA or EMEA. Using recombinant lysozyme involves high raw material costs and analytical efforts. Thermal treatment of the cells is another option for a disintegration technique that avoids shear forces, as described in

WO 02/057446 A2 and WO 96/36706. The suspension of microorganisms processed by short time exposure (30 seconds to some minutes) to 80°C in a sink heater or in a filter (with filtering aids). This method is usually carried out in combination with a detergent (e.g. TRITON® ~~Triton®~~) and lysozyme.

At pages 5-6 of the filed application, replace paragraph [0017] (corresponding to paragraph [0022] of the published application) with the following new paragraph.

During alkaline lysis, cells are subjected to an alkaline solution (preferably NaOH) in combination with a detergent (preferably SDS). In this environment, the cell wall structures are destroyed thereby releasing the polynucleotide of interest and other cell related compounds. Finally, the solution is neutralized by addition of a solution of an acidic salt, preferably an acetate, in particular potassium acetate (KAc) or sodium acetate (NaAc). The alkaline conditions lead to denaturation of pDNA by unwinding the supercoiled structure. Up to a pH-value of 12 to 12.5 the complete separation of the complementary strands is prevented. This enables entire renaturation of the plasmid molecule, when the pH is decreased again. If the pH-value exceeds the renaturation limit, the unseparated regions are lost and the pDNA is irreversibly denatured. At this stage the polynucleotide contains large domains of single stranded material (with a large exposure of hydrophobic bases) (Diogo MM, Queiroz JA, Monteiro GA, Prazeres DMF (1999) Analytical Biochemistry 275:122). The exact pH-value for irreversible denaturation of the plasmid is strongly influenced by the base pair composition, the resulting hydrogen bonds and its size (WO 97/29190). In parallel, genomic DNA and proteins are denatured,

too. Denaturation of DNA leads to entanglement and formation of long single pair strands with low mechanical stability. Impact of mechanical stress may cause breakage of DNA, especially of the large gDNA molecules. The resulting fragments have properties comparable to those of pDNA. Since precipitation during the subsequent neutralization step is a size dependent process, these fragments may remain soluble and thus behave similarly to pDNA (Marquet M, Horn NA, Meek JA (1995) BioPharm September:26). Therefore they would interfere during the isolation process. The incubation time at high pH value is critical for the renaturation of the target polynucleotide, the degree of cell disintegration and the genomic DNA content in the preparation. Therefore the main parameter for quality and quantity of the polynucleotide preparation is the contact time with the alkaline lysis solution. Usually RNAse is added to the suspension to digest RNA into small pieces not to interfere the isolation process (Sambrook J, Fritsch EF, Maniatis T, (1989) Molecular Cloning: A Laboratory Handbook, CSH Press, Cold Spring Harbor, USA). After addition of NaOH and SDS, the solution becomes highly viscous. Local pH extremes, which irreversibly denature the plasmid (Rush M G, Warner R C (1970) J Biol Chem 245:2704) have to be avoided. Fast and efficient mixing has to be guaranteed in order to achieve a homogenous solution. Usually small containers like glass bottles containing the viscous solution are mixed very gently by hand (QIAGEN® ~~Qiagen®~~ Plasmid-Handbuch January 2001, Qiagen GmbH, Germany). This procedure can only be performed in a batchwise mode with a maximum of about 5 l lysate per bottle. It is mainly operator dependent, providing low reproducibility and is therefore not suited for a manufacturing scale. For large scale conventional stirrers are not suited because they may cause damage to pDNA and

gDNA. Some processes use optimized tanks and stirrers or a combination of different mixers in order to overcome these problems (Prazeres DMF, Ferreira GNM, Monteiro GA, Cooney CL, Cabral JMS (1999) Trends Biotechnol 17:169; WO 02/26966).

At pages 8-9 of the filed application, replace paragraph [0023] (corresponding to paragraph [0028] of the published application) with the following new paragraph.

For disintegration (lysis) of the cells in view of obtaining polynucleotides, several different methods have been suggested, e.g. methods that use thermal or chemical treatment. For the thermal lysis, a process using a flow-through heat exchanger (70-100° C), in which the cells are continuously disintegrated after incubation of the resuspended cells in presence of a detergent and optionally lysozyme, is described (WO 96/02658 A1). Another physical method, which works in a temperature range of 70-90°C, is shown in WO 02/057446 A2:In a first step, the harvested cells are filtered utilizing filter aids and the resulting mixture is thermally lysed in a second step. Alternatively, disintegration can be carried out by pumping hot lysis buffer through the filter cake or by a flow through heat exchanger. Chemical lysis methods are operated at an alkaline pH-value, they are therefore referred to as "alkaline lysis". A commonly used composition of the intrinsic lysis solution is described by Birnboim and Doly, but there are exist many variants of this solution. As the detergent that is part of lysis solution usually SDS is used, but other (e.g. non-ionic) detergents like TWEEN® or TRITON® ~~Tween® or Triton®~~ are also suitable (e.g. WO 95/21250 A2). According to EP 0376080 A1, SDS is replaced by desoxycholate (DOC), while the three phase extraction method of U.S. Pat.

No. 5,637,687 uses a novel composition for the cell-solubilization (benzyl alcohol+sodium iodide+guanidinium thiocyanate and/or guanidinium chloride). Most methods for alkaline lysis are operated in a batchwise mode. By way of example, the alkaline treatment can be carried out directly by adding a NaOH/SDS solution to a bacterial cell culture during exponential growth (in this case, no harvest of the cells is performed) or after resuspension of the cells in a proper buffer. Thereby, an alkaline solution is added until a pH value is reached that is 0,2 units lower than the pH value at which the pDNA-molecules are completely denatured, a pH value that is empirically determined and different for each single plasmid (WO 97/29190 A1). Another method utilizes a column comprising a carrier on a membrane filter that is capable of retaining a solution and permeating it by aspiration. When adsorbed onto the carrier, a certain amount of cells can be lysed in this column by means of lysozyme and further processed (EP 0814156 A2). A similar device that consists of a hollow body (tube) with a built-in filtration-layer is disclosed in EP 0616638 B1, EP 0875271 A2, and WO 93/11218 A1. Alkaline lysis is carried out in the part of the tube above the filtration section. The cell suspension and the used solutions are distributed and mixed in a non-continuous way.

At page 14 of the filed application, replace paragraph [0035] (corresponding to paragraph [0040] of the published application) with the following new paragraph.

Furthermore, as a pretreatment before the final purification, addition of an "Endotoxin ~~Endotoxine~~ Removal (ER) Buffer" (QIAGEN® ~~Quiagen®~~) (WO 00/09680 A1) or TRITON X® ~~Triton X®~~-114 (WO 99/63076 A1) has been suggested.